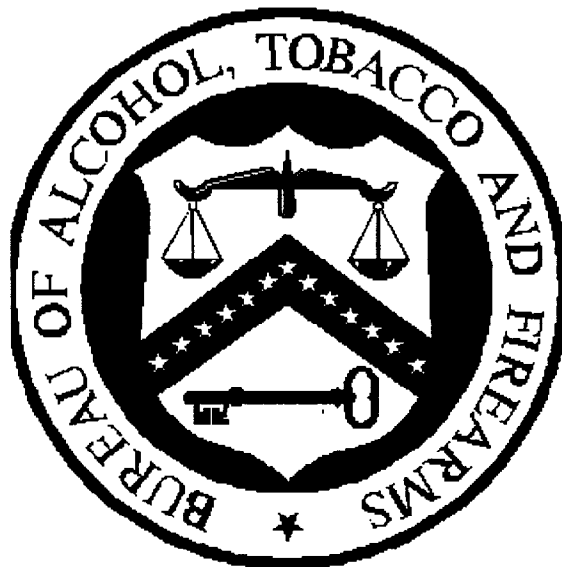


Department of the Treasury  
Bureau of Alcohol, Tobacco & Firearms

# REQUIRED ANALYTICAL TESTS FOR WINERIES



# “Required Analytical Tests for Wineries”

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## **Objective:**

*At the end of this course, participants will be able to perform the analyses demonstrated by ATF.*

## **Contents:**

Sulfur Dioxide Determination.....	3
Wine Alcohol Determination.....	10
Fill of Wine Containers.....	19
Wine Acidities .....	22
Other Information.....	30

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## SULFUR DIOXIDE

Federal regulations for the Labeling and Advertising of Wine (27 CFR Part 4) limit the amount of total sulfur dioxide to 350 mg/L (ppm) in the finished wine (27 CFR 4.22(b)(1)). Also, section 4.32(e) requires that a sulfite declaration appear on wine and other beverage alcohol products containing 10 or more mg/L total sulfur dioxide, regardless of whether any sulfiting agents were added to the grapes, juice or wine. Furthermore, section 27 CFR 24.309(m) of the Federal Wine Regulations requires that information appear on the transfer in bond record (formerly ATF Form 703) about any substance for which a label declaration is required for the finished product.

### METHODS FOR DETERMINING SULFUR DIOXIDE

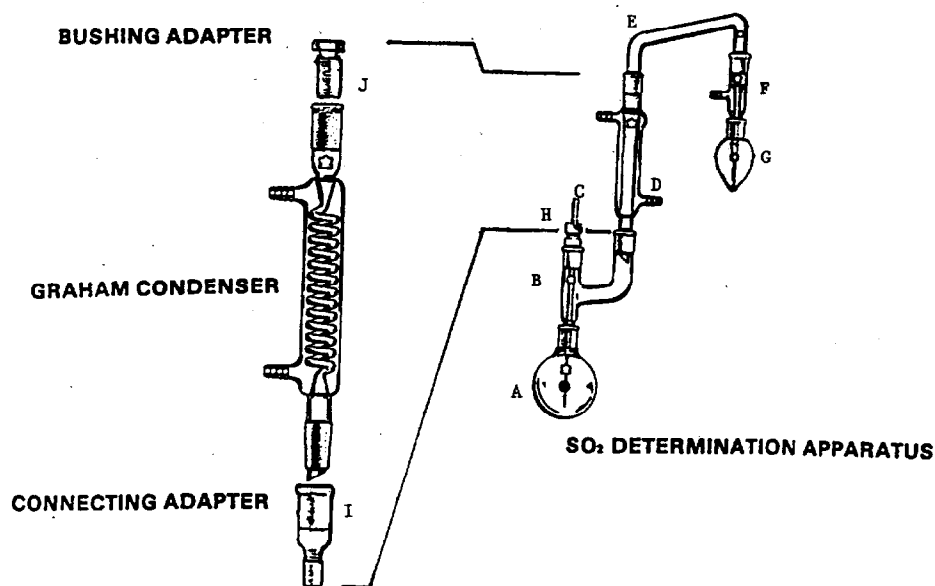
<u>Analysis:</u>	<u>Ripper</u>	<u>Aeration-Oxidation</u>
<u>Suitability</u>		
Free S02	yes	yes
Total S02	yes	yes
Relative ease of analysis	easy	medium
Initial cost	\$80	\$250
Time min/analysis	5/15	15
Significant interferences	yes	rare
Ease of end-point:		
white	easy	easy
red	difficult	easy

## DETERMINATION OF SULFUR DIOXIDE IN WINES BY AERATION/OXIDATION

### Equipment

1. Distillation-scrubber apparatus (all 14/20 standard taper pieces, referenced as Kontes part numbers):

- A. 250 mL round-bottom flask, K 294000-0250
  - B. Claisen adapter K 273750
  - C. Pasteur pipette through rubber stopper
  - D. 300 mm Graham condenser K 439000-2430
  - E. Connecting adapter K 275050
  - F. Vacuum Adapter K 276750
  - G. Two 50 mL pear flask K 294250-0050
  - H. Inlet adapter, size 21 K 179800-2114
  - I. Connecting adapter, 24/40 - 14/20 K 274750-0263
  - J. Bushing adapter, 24/40 - 14135 K 150750-0242
2. 10 mL Burette
  3. Vacuum source/water aspirator
  4. 5, 10 and 20 mL volumetric pipettes
  5. Heat shrink tubing



Assemble as shown. Connect Pasteur pipette inlet as described above. Connect a recirculating ice bath to the Graham condenser and place a micro-burner below the round bottom flask.

**Reagents:**

1. 0.01 N sodium hydroxide solution: purchase pre-mixed or prepare by dissolving 0.41 g NaOH in 500 mL distilled water and diluting to 1 L
2. Standardized 0.01 N sulfuric acid (for calibrating the sodium hydroxide solution).
3. 0.3% hydrogen peroxide solution: prepare just prior to use by diluting 10 mL reagent grade 3% hydrogen peroxide solution to 100 mL with distilled water.
4. Indicator: dissolve 0.100 g methyl red and 0.05 g methylene blue in 50% aqueous ethanol and make to 100 mL.
5. 25% phosphoric acid solution: add 280 mL 90% phosphoric acid to 800 mL distilled water; cool; make to 1 L.

**Standardization Procedure:**

1. Add 10 mL distilled water and 3 drops of indicator to the pear flask; adjust the purple color of the solution to turquoise green by adding small drops of sodium hydroxide solution; remember the color.
2. Pipette 5 mL of standard sulfuric acid solution into the flask; swirl.
3. Fill the burette to the zero mark and titrate the solution to the same green color as noted in step 1; record the titer.
4. Repeat the standardization until 3 titers agree within 0.05 mL. This is also excellent practice in recognizing the endpoints,
5. The base normality is  $0.01 \times 5 / (\text{average mL base used in standardization})$ .

### Sample Determination:

1. Add 10 mL hydrogen peroxide solution and 3 drops of indicator to the pear flask; adjust the purple color of the solution to turquoise green by adding small drops of sodium hydroxide solution and attach the flask to the end of the vacuum adapter.
2. a) Total  $\text{SO}_2$ : Pipette 10 mL wine and 10 mL phosphoric acid solution into the 50 mL pear shaped flask. If the wine is gassy or foamy, add a drop of anti-foam. Attach the flask to the end of the Claisen adapter,  
  
b) Free  $\text{SO}_2$ : Pipette 100 mL wine + 20 mL  $\text{H}_2\text{PO}_4$  into 250 mL round bottom flask. Draw air through vacuum adapter at 200 mL/min for 15 minutes.
3. For total sulfur dioxide: draw air through the vacuum adapter at 200 mL/min while gently boiling the pear flask with a micro-burner for 15 minutes.
4. Carefully remove the pear flask, rinsing the tip of the vacuum adapter into the flask.
5. Fill the burette to the zero mark and titrate the solution to a turquoise green color; record the titer.

### Calculation:

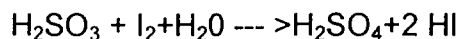
$$\text{SO}_2 \text{ (free/total) mg/L} = \frac{(\text{mL base titer}) \times 32000 \times (\text{normality base})}{10 \text{ (for total) or } 100 \text{ (for free)}}$$

### Reporting Results:

Report values as mg/L (ppm) with no decimals, i.e., XXX mg/L (ppm).

## DETERMINATION OF SULFUR DIOXIDE IN WINE BY THE RIPPER METHOD

### Reaction Equation:



### Equipment:

1. 250 mL Erlenmeyer Flask
2. 25/10 mL Buret and holder
3. 20/50 mL volumetric pipet for sample
4. 5, 10, & 25 mL pipettes for starch, NaOH, and H<sub>2</sub>SO<sub>4</sub>
5. Stopper or parafilm
6. Titration Light Source

### Reagents:

1. Sulfuric acid solution: 1+3 (10 mL sulfuric acid 30 mL water)
2. 1 N sodium hydroxide solution (40 grams NaOH per liter water): for total SO<sub>2</sub>
3. 2% starch indicator solution
4. Iodine: 0.020 N, standardized against sodium thiosulfate solution.  
Note: Iodine solution should be stored in dark colored glass containers. Also, iodine oxidizes easily and should be standardized frequently,
5. Standard sodium thiosulfate solution

### Procedure: (Free)[Total] Sulfur Dioxide:

1. Pipette (50)[20] mL of wine into a 250 mL Erlenmeyer flask.
  - 1.a. For total sulfur dioxide only: [Add 25 mL of sodium hydroxide solution; mix, stopper, and let stand for 10 minutes].
2. Add 5 mL starch indicator solution; add (5)[10] mL sulfuric acid solution.
3. Titrate rapidly with 0.020 N iodine solution; endpoint is the first bluish color persisting for about 30 seconds.

**Calculations:**

$$SO_2 \text{ [free/total]}(\text{mg/L}) = \frac{(\text{mL iodine}) \times (\text{N iodine}) \times (32000)}{\text{mL sample}}$$

Example: 20 mL sample uses 4.6 mL of 0.020 N iodine, then  
 $SO_2 = (4.6) \times (0.020) \times (32000) / 20 = 147 \text{ mg/L}$

**References:**

1. Ough, C.S. and Amerine, M.A., Methods for Analysis of Musts and Wines, J. Wiley and Son, New York (1988).
2. Zoecklin, S.W., Fugelsang, K.C., Gump, B.H. and Nury, F.S., Production Wine Analysis, Van Nostrand Reinhold, New York (1 990).



## SULFITE CALCULATIONS FOR POTASSIUM METABISULFITE (KMBS) ADDITIONS

### A. Weight of Fruit

$$\frac{\text{pounds of fruit}}{2.2 \text{ lbs. per kilogram}} \times \frac{\text{\#\# ppm SO}_2}{1,000} \times 1.74 = \text{grams KMBS to add}$$

→ Factor: KMBS is 57.5% SO<sub>2</sub>  
 (1/0.575 = 1.74)

→ Desired addition level of SO<sub>2</sub> in mg/L (ppm). Must convert mg to grams since equation determines the number of grams of KMBS to add.

→ Convert pounds to kilograms

Example:

How many grams of KMBS are needed to add 50 ppm SO<sub>2</sub> to 750 pounds of grapes?

$$\frac{750 \text{ pounds}}{2.2} \times \frac{50}{1,000} \times 1.74 = 30 \text{ grams KMBS}$$

### B. Volume of Wine or Juice

$$3.785 \times \text{gallons} \times \frac{\text{Increase in SO}_2 \text{ (mg/L)}}{1,000} \times 1.74 = \text{grams of KMBS}$$

→ Factor: KMBS 57.5% SO<sub>2</sub>

→ Desired increase in SO<sub>2</sub> in mg/L (ppm). Must convert mg to grams (divide by 1,000) since equation determines the number of grams of KMBS to add.

→ Convert gallons to liters

Example:

How many grams of KMBS are required to increase the SO<sub>2</sub> level in 400 gallons of wine from 10 ppm to 30 ppm?

$$3.785 \times 400 \text{ gals} \times \frac{(30-10)}{1,000} \times 1.74 = 53 \text{ grams KMBS}$$

## WINE ALCOHOL

Following water, ethyl alcohol is the second major constituent of wine. Alcohol is very important because it makes significant contributions to both the sensory properties and the stability of wine.

Wine is defined in the Federal Alcohol Administration Act as containing not less than 7 percent and not more than 24 percent alcohol by volume (27 U.S.C. 211 (a)(6)). Internal Revenue Code regulations define wine as containing not less than 0.5 and not more than 24 percent alcohol by volume (27 CFR 24.10). Federal labeling regulations require that wines be labeled with an alcohol representation within a  $\pm 1.5$  percent tolerance for wines between 7 and 14 percent alcohol and a tolerance of  $\pm 1.0$  percent alcohol by volume for wines over 14 percent alcohol by volume (27 CFR 4.36). There is a tolerance of  $\pm 0.75$  percent by volume for wines with less than 7 percent alcohol by volume stated on the label (27 CFR 24.257(a)(4)).

Still wine products containing between 0.5 and 14 percent alcohol by volume are taxed at the rate of \$1.07 per gallon. Wine products above 14 percent and not over 21 percent alcohol by volume are taxed at the rate of \$1.57 per gallon (26 U.S.C. 5041). Wineries producing less than 250,000 gallons in a calendar year may receive a tax credit of up to \$0.90 per gallon on the first 100,000 gallons of wine removed annually for consumption or sale (27 CFR 24.278).

Since wine is taxed on alcohol content and wine labels must accurately reflect the alcohol content of wine, accurate alcohol determinations are important. Additionally, Federal regulations require wineries producing or processing bulk wine to have ready access to a suitable means of determining the alcohol content of wine (27 CFR 24.278). Section 24.355(e) requires wineries to conduct alcohol tests of wine bottled or packed, and section 24.308(c) requires wineries to maintain records of these alcohol tests.

## DETERMINATION OF ALCOHOL IN WINE BY DISTILLATION/HYDROMETRY

### Introduction:

Distillation converts wine into an alcohol-water solution of the same concentration as the original wine. The alcohol concentration of this distillate may be determined by density (hydrometry), refractometry, or, if the original wine solids were too high, an ebulliometer.

### Equipment:

One of the following hydrometers of appropriate range with calibrated corrections:

- Indicating specific gravity with divisions of 0.0001
- Indicating proof with divisions of 0.2°
- Indicating percent alcohol with divisions of 0.1%

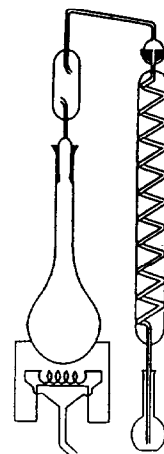
Thermometer with calibrated corrections with divisions of 0.2°F

Conversion Table (depending on the hydrometer used):  
Table 1 of the "Gauging Manual" (ATF P 5110.6), *or*  
Table 52.003 of Official Methods of Analysis of the  
A.O.A.C. (14th ed) (or equivalent table), *or*  
Alcohol Correction Table from Bureau of Internal  
Revenue, Bulletin 7 (1937) (*attached*)

Hydrometer cylinder - clear glass 2.5" diameter

250 ml volumetric flask with stopper

Distillation apparatus - electric or gas operated - with:  
Distillation flask, Condenser and Kjeldahl Connector  
Constant temperature water bath [maintained at  
20°C (68-F) ± 0.1° C]  
Boiling chips



**Reagents:** Antifoam agent, Distilled water, Ice  
2 N aqueous sodium hydroxide (NaOH) solution

**Distillation:**

1. Fill the 250 mL volumetric flask to above the graduation mark with the wine sample and stopper.
2. Temper at 20°C for one hour, adjust wine volume to graduation mark.
3. Quantitatively transfer the entire contents of the flask to a distillation flask. Rinse the volumetric flask at least 3 times using a total of 50 to 60 mL of distilled water, adding all of the rinse water to the distillation flask.
4. Newly fermented, gassy, or wines in excess of 5 percent sugar, may require the addition of a drop of antifoam agent to the contents of the distillation flask to prevent excessive foaming.
5. Wines containing in excess of 200 mg/L of sulfur dioxide or 0.1 g/100 mL volatile acidity must be neutralized with two drops of 2 N NaOH prior to distillation to avoid interferences resulting from the presence of these materials.
6. Position and connect the distillation flask ensuring a tight seal between the flask and the trap, and the trap and the condenser.
7. Place the rinsed receiving flask in a beaker of ice water so that it will be immersed nearly to the stem during the distillation period. The outlet of the condenser should extend into the neck of the flask, but should not touch the surface of the distillate.
8. Pass a continuous stream of cold (<25°C) water rapidly through the water jacket of the condenser.
9. Heat the flask rapidly until the liquid reaches its boiling point, then distill at a uniform rate, completing the distillation in from 30 to 60 minutes, depending upon the alcohol content of the wine sample.

10. Collect approximately 225 mL of distillate, stopper, and again temper for one hour at 20°C.

11. Bring volumetric flask up to volume using tempered (20°C) distilled water. Stopper and mix thoroughly.

12. Temper flask at room temperature for one hour.

**Determination:**

13. Transfer distillate to dry hydrometer cylinder containing thermometer, and immerse a dry hydrometer of appropriate range.

14. After hydrometer reaches equilibrium, read hydrometer to nearest:

0.02° for proof indicating hydrometers

0.01% for % alcohol indicating hydrometers

0.0002 units for specific gravity indicating hydrometers

Record temperature of distillate to nearest to 0.02°F. Correct the hydrometer and thermometer readings.

**Calculation:**

Percent alcohol-indicating hydrometers: convert the % alcohol at the measured temperature to 60°F via the table in Bulletin 7.

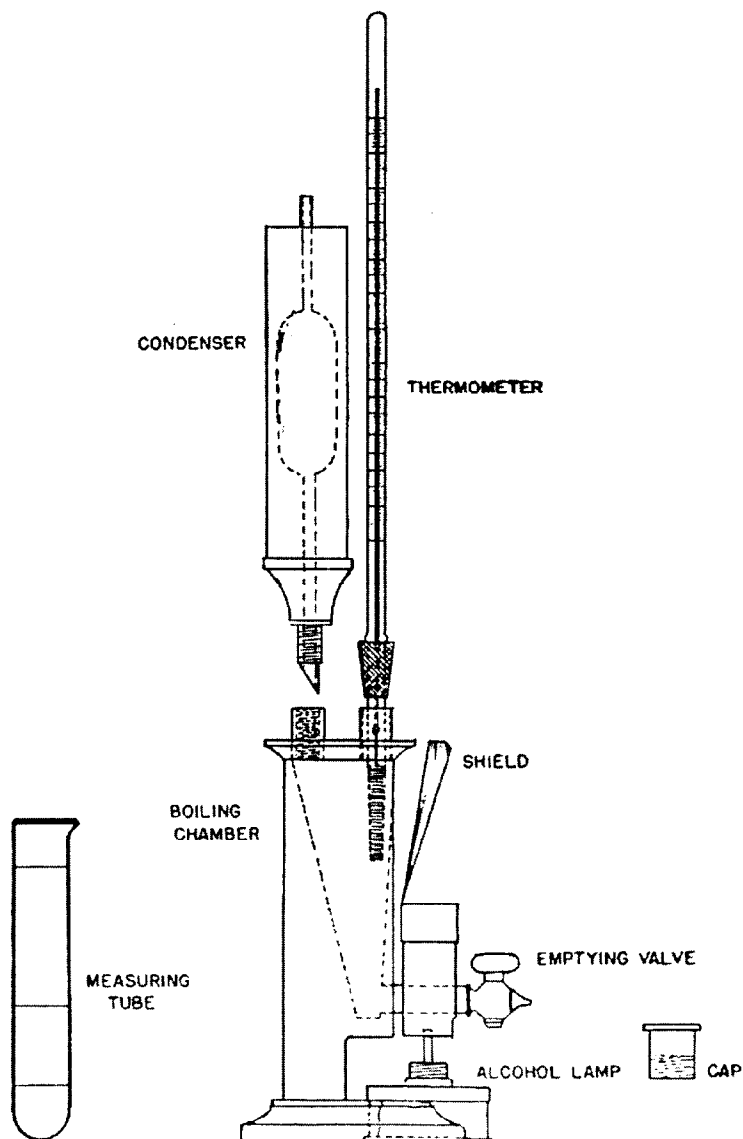
Proof-indicating hydrometers: convert the proof at the measured temperature to 60°F via the Gauging Manual and divide the resulting proof by 2.

Specific gravity-indicating hydrometers: convert the specific gravity at the measured temperature to 60°F via A.O.A.C. Table 52.003. (Some interpolation may be necessary to use this table.)

This method is accurate to  $\pm 0.1\%$ . Report results to 1 decimal place.

**Equipment:**

- Ebulliometer
- Slide rule/conversion table
- Alcohol/micro bunsen burner
- 200 mL volumetric flask with stopper
- 50 mL pipette
- 100 mL graduated cylinder
- Narrow range ebulliometer thermometer



**Reagents:** antifoam solution and distilled water

**Procedure:**

1. Bring the sample and a supply of distilled dilution water to a temperature of 20°C (68°F).
2. Using a graduated cylinder, transfer the required amount of distilled water to the boiler. Insert the thermometer. No water is added to the condenser for the determination of the boiling point of water.
3. Protect the ebulliometer from drafts.
4. Ignite micro-burner/alcohol lamp. Adjust flame (which may require trial runs) to start the temperature rise within 8-9 minutes.
5. Observe temperature rise, noting (writing) temperatures every 15 seconds; continue reading temperature until 3 successive readings are the same. (Note that the smallest graduation is 0.05°C; estimate readings to 0.01°.)
6. Remove flame. Remove thermometer. Drain the HOT water carefully.
7. Transfer 50 mL of the sample to a 200 mL volumetric flask (by pipette) and make up to volume with distilled water, stopper & mix.
8. Rinse boiler 3 times with approximately 25 mL of diluted sample. Transfer by graduated cylinder the required amount of diluted sample to boiler. Attach condenser firmly. Insert thermometer. Add cold (ice) water to condenser jacket. Put finger over condenser vent tube to prevent water entering the boiler.
9. Light flame: adjust flame to attain temperature rise within 8-10 minutes. Observe and record every 15 seconds until temperature stabilizes (three consecutive readings are the same). Repeat the determination if condenser gets warm.
10. Remove flame; remove thermometer, drain sample; drain condenser.

11. Repeat steps 7-10 to replicate analysis or for new sample.
12. Calculate the alcohol from the slide rule/conversion table that comes with the instrument; multiply the alcohol found by the dilution factor.

**Notes:**

- The ebulliometer and glassware should be "squeaky" clean internally as well as externally, and the work area should be orderly and free of miscellaneous equipment.
- Clean the boiler by boiling dilute alkaline solution every 25-30 determinations.
- If you have access to a known alcohol standard solution (such as 80 proof [40% alcohol] gin) you will be better able to evaluate the performance of your equipment and techniques.
- To prevent loss of alcohol, reflux condensers must be securely tightened into the lower housing and the thermometer must also be sealed at the housing.
- Cooling water in the condenser must be as cold as possible.
- In newly fermented wines, excessive foaming may be prevented by the addition of antifoaming agents.
- The marked tubes for diluting samples frequently furnished with the Instrument are adequate for approximate work; for best precision use class A pipettes calibrated to deliver (T.D.), and class A volumetric flasks calibrated to contain (T.C.); both at 20°C (68°F).
- Do not blow out pipettes that are calibrated to deliver. As the last bit of sample flows from the tip, touch the end of the pipette briefly against an interior surface for proper drainage.



- Do not exceed a temperature difference of 4°C between the boiling points of water and the sample. Wine should be diluted to 4 or 5% alcohol; 1:4 dilution is even better (50 mL wine aliquot diluted to 200 mL with distilled water).
- Sugar levels in excess of 2 percent as well as aldehydes and esters depress the boiling point of the sample resulting in higher apparent alcohol levels. To minimize these errors, sweet wines can be quantitatively diluted with distilled water to less than 2% sugar or distilled.

**References:**

1. Ough, C.S. and Amerine, M.A., Methods for Analysis of Malts And Wines, J. Wiley and Son, New York (1988).
2. Official Methods of Analysis of the A.O.A.C. International, Arlington, VA
3. Zoecklin, S.W., Fugelsang, K.C., Gump, S.H. and Nury, F.S., Production Wine Analysis, Van Nostrand Reinhold, New York (1990).

## ALCOHOL CORRECTION FACTORS (% v/v) FOR TEMPERATURES ABOVE OR BELOW 60°F (15.5°C)

Alcohol	To or from the observed																							
	Add												Subtract											
Content	At 57°F	At 58°F	At 59°F	At 61°F	At 62°F	At 63°F	At 65°F	At 66°F	At 67°F	At 68°F	At 69°F	At 70°F	At 72°F	At 74°F	At 76°F	At 78°F	At 80°F							
percent	percent	percent	percent	percent	percent	percent	percent	percent	percent	percent	percent	percent	percent	percent	percent	percent	percent							
1	0.14	0.10	0.05	0.05	0.10	0.16	0.22	0.28	0.34	0.41	0.48	0.55	0.62	0.77	0.93	1.10	1.28							
2	.14	.10	.05	.05	.11	.17	.23	.29	.35	.42	.48	.56	.63	.78	.94	1.10	1.28							
3	.14	.10	.05	.06	.12	.18	.24	.30	.36	.43	.50	.57	.64	.80	.96	1.13	1.31							
4	.14	.10	.05	.06	.12	.19	.25	.32	.38	.45	.52	.59	.67	.83	1.00	1.17	1.35							
5	.15	.10	.05	.07	.13	.20	.26	.33	.40	.47	.54	.62	.70	.86	1.03	1.21	1.40							
6	.17	.11	.06	.07	.14	.20	.27	.34	.42	.50	.57	.66	.74	.90	1.09	1.27	1.46							
7	.18	.12	.06	.07	.14	.21	.29	.36	.44	.52	.60	.68	.77	.94	1.13	1.32	1.52							
8	.19	.13	.06	.08	.16	.23	.31	.39	.47	.55	.64	.73	.81	.99	1.18	1.38	1.59							
9	.21	.14	.07	.08	.16	.24	.32	.41	.50	.58	.67	.76	.86	1.04	1.25	1.46	1.67							
10	.23	.16	.08	.08	.17	.25	.34	.43	.52	.61	.71	.80	.90	1.10	1.32	1.54	1.76							
11	.25	.16	.08	.09	.18	.27	.37	.46	.56	.65	.75	.85	.96	1.16	1.39	1.61	1.84							
12	.27	.18	.09	.10	.20	.29	.39	.49	.59	.70	.80	.91	1.02	1.23	1.46	1.70	1.94							
13	.29	.19	.10	.10	.21	.31	.42	.52	.63	.74	.85	.97	1.08	1.31	1.55	1.80	2.05							
14	.32	.21	.11	.11	.22	.32	.44	.55	.66	.78	.91	1.02	1.14	1.39	1.65	1.91	2.17							
15	.35	.23	.12	.12	.24	.35	.48	.60	.71	.84	.97	1.10	1.23	1.50	1.76	2.03	2.30							
16	.37	.24	.12	.13	.26	.38	.52	.65	.77	.90	1.03	1.17	1.31	1.60	1.88	2.16	2.44							
17	.40	.26	.13	.14	.27	.41	.54	.68	.82	.96	1.10	1.25	1.40	1.70	2.00	2.28	2.57							
18	.44	.29	.14	.14	.29	.44	.58	.73	.88	1.03	1.18	1.33	1.49	1.80	2.10	2.41	2.72							
19	.47	.32	.16	.15	.30	.46	.62	.78	.94	1.10	1.26	1.42	1.58	1.90	2.22	2.54	2.86							
20	.51	.34	.17	.16	.32	.49	.66	.82	.98	1.15	1.33	1.48	1.65	2.00	2.32	2.65	3.00							
21	.53	.35	.18	.17	.34	.51	.68	.85	1.02	1.20	1.38	1.54	1.72	2.06	2.41	2.76	3.10							
22	.56	.38	.19	.17	.36	.53	.71	.90	1.07	1.25	1.44	1.61	1.78	2.13	2.48	2.84	3.18							
23	.58	.40	.20	.18	.37	.55	.74	.92	1.11	1.30	1.49	1.66	1.84	2.20	2.56	2.93	3.27							
24	.60	.40	.20	.18	.38	.56	.77	.96	1.16	1.35	1.54	1.72	1.91	2.27	2.65	3.03	3.40							

Source: Bureau of Internal Revenue, Bulletin 7, 1937

## FILL OF WINE CONTAINERS

Section 27 CFR 24.255(b) of the Federal Wine Regulations requires, in part, that bottles and other containers be filled as nearly as possible to conform to the net contents as shown on the label; but in no event may the amount of wine contained in any individual container, due to lack of uniformity of containers, vary from the amount stated on the label more than:

- 1.0% for 15.0 liters and above
- 1.5% for 1.0 to 14.9 liters
- 2.0% for 750 mL
- 2.5% for 500 mL\*
- 3.0% for 375 mL
- 4.5% for 187 mL and 100 mL
- 9.0% for 50 mL

*(\*Acceptable tolerance; not listed in regulation)*

In such case, there will be substantially as many bottles overfilled as there are bottles underfilled for each lot of wine bottled. A "lot of wine bottled" is defined as the same type of wine bottled or packed on the same date into containers (27 CFR 24.10).

27 CFR 24.255(d) requires that fill tests be conducted at representative intervals of wine bottled or packed during the bottling or packing operation for each bottling or packing line to determine if the wine contained in the bottle is in agreement with that stated on the label.

27 CFR 24.308(c) requires, in part, that records of fill tests be maintained for each lot of wine bottled or packed, or for each bottling or packing line operated each day, showing the date, type of test, item tested and results.

27 CFR 24.170(b) requires, in part, that bottling or packing wineries have ready access to equipment for determining the net contents of bottled or packed wine. Fill tests may be conducted either volumetrically or by weight.

## DETERMINATION OF FILL OF CONTAINERS BY WEIGHT

### Equipment:

1. Top loading balance with 0.1 g readability
2. Specific gravity hydrometer
3. Thermometer
4. Pycnometer or volumetric flask

### Procedure:

For containers which have already been filled:

1. Weigh full bottle ( $W_o$ ), noting the labeled capacity of the container ( $V_o$ ). If the sample is highly carbonated, carefully open the container and let it de-gas for 20 minutes.
2. Empty the contents and let the container thoroughly drain.
3. Reweigh the drained container ( $W_e$ ), including the closure.
4. Determine the density ( $D$ ) of the contents, noting the temperature. Be sure that carbonated products are thoroughly degassed.

For containers on the filling line:

1. Weigh a marked container ( $W_e$ ), noting the label capacity of the container ( $V_o$ ); a group of containers may be run at the same time.
2. Obtain a bulk sample of the product from the bottling tank and determine the density ( $D$ ), noting the temperature. Be sure carbonated products are thoroughly degassed.
3. Run the container(s) through the filler and recover them. Determine full weight of the container ( $W_o$ ), noting the labeled capacity of the container ( $V_o$ ). If the sample is highly carbonated, let it de-gas for 20 minutes before weighing.

**Calculations:**

$$\text{Fill (as \% of label volume)} = \left[ \frac{(W_o - W_e) - V_o}{D} \right] \times \frac{100}{V_o}$$

[Fill is reported as + or - % of label contents with one decimal place, i.e., + or - X.X %].

## WINE ACIDITIES

### Introduction:

Wine acid is very important as it contributes to the color, stability and sensory properties of wine. Tartaric and malic acid are the two major acids in grape wines and account for about 90% of a grape wine's total titratable acidity. Occasionally, winemakers add acids to must or wine to compensate for natural deficiencies and also for stabilization purposes.

The Federal Wine Regulations (27 CFR Part 24) provide for the addition of tartaric and/or malic acids to grape must prior to fermentation and any one or combination of the following acids to grape wine after fermentation: Tartaric, malic, citric, lactic and fumaric to compensate for naturally occurring deficiencies (27 CFR 24.182(b)). However, this same section of the regulations states that the uses of these acids, either prior to, during or after fermentation, may not increase the fixed acidity (fixed being simply the difference between the total [titratable] acidity and the volatile acidity) of the finished wine (calculated as tartaric) above 9.0 grams per liter.

In cases where the wine contains 8.0 or more grams of total solids per 100 mL of wine, acids may be added to the extent that the finished wine does not contain more than 11.0 grams per liter of fixed acid (calculated as tartaric).

In fruit (other than grape) wine production, 27 CFR 24.182(c) provides that only citric acid may be added to citrus fruit, juice or wine, only malic acid may be added to apple fruit, juice or wine, and only citric acid or malic acid can be added to other fruit or to juice or to wine derived from other fruit (including berries) to correct for natural deficiencies to 9.0 grams per liter of finished wine; however if the wine contains 8.0 or more grams of total solids per 100 ml of wine, acids may be added to correct for natural deficiencies to the extent that the finished wine does not contain more than 11.0 grams per liter of fixed acid (calculated as malic acid for apples and citric acid for other fruit, including berries).

27 CFR 24.244 provides for the addition of up to 0.7 grams per liter of citric acid, regardless of the fixed acidity level, to stabilize wine. Citric acid chelates iron present in wine and helps prevent iron instabilities. The same section of the regulations provides for the addition of up to 3.0 grams per liter of umaric acid, regardless of the fixed acidity level, to impede malo-lactic fermentation.

27 CFR 24.318 requires that wineries which add acid to wine shall maintain records showing the date of use, the kinds and volume of wine in which used, and, when used to correct natural deficiencies, the fixed acid level of the juice or wine before and after the addition. The record will account for all acids received and will be supported by purchase invoices.

Volatile acid at high levels is often regarded as an indication of spoilage. Federal regulations provide limitations on the amount of volatile acidity in standard wine. Section 27 CFR 4.21(a)(1)(iv) of the Federal regulations for the Labeling and Advertising of Wine provides a maximum volatile acidity limitation, calculated as acetic acid exclusive of sulfur dioxide, of 0.14 g/100mL for natural red wine and 0.12 g/100 mL for other grape wine. However, the maximum volatile acidity for wines produced from unameliorated juice of 28 or more degrees Brix is 0.17 g/100mL for red wine and 0.15 g/100mL for white wine.

**Equipment:**

10 mL burette [marked in 0.1 mL]  
1, 5 and 10 mL pipettes  
Low vacuum source or water aspirator  
A source of condenser water  
Cash still  
(pH meter with combination reference/pH electrode – optional)  
Magnetic stirring table and stir bar  
250 mL beaker

## DETERMINATION OF TITRATABLE (TOTAL) ACIDITY

1. Standardize pH meter using pH 10.0 and pH 7.0 buffers.
2. Rinse electrodes with distilled water.
3. Add enough distilled water to a 250 mL beaker so that electrode end will be sufficiently submersed when lowered into the beaker.
4. Place electrode into the beaker and adjust to pH 8.2 using 0.1 N or less NaOH. This neutralizes any acid which may be present in the distilled water and need not be a quantitative addition. Place electrode away from the magnetic stir bar to prevent possible damage to electrode. If a phenolphthalein indicator end point is used in lieu of a pH meter, add 2 or 3 drops of a 1% phenolphthalein solution to the distilled water. Titrate with 0.1 N or less NaOH to a faint pink color.
5. Pipette exactly 5.0 mL of degassed wine sample into the same beaker using a T.C. (to deliver) pipette. Do not blow-out or otherwise attempt to remove any of the wine residue from the pipette as these pipettes are calibrated "to deliver" their rated capacity.
6. Titrate the water-wine solution using 0.1 N NaOH, slowly allowing the titrant and the sample to mix. As the pH approaches 8.2, add the titrant very slowly as to not over shoot the pH 8.2 end point. In a phenolphthalein indicator is used, add about 1 mL of phenolphthalein solution to the distilled water-wine solution and titrate using 0.1 N NaOH to a faint pink color end-point. Three or four samples may be analyzed using the same buffering solution prepared in steps 3 and 4 above.
7. Record the quantity of NaOH used in the titration.



**Calculation:**

Report results in g/L using the following formula:

Total Acid: (as appropriate acid in g/L)

Grape wines: g/L tartaric = mL NaOH x N NaOH x 0.075 x 200

Apple wines: g/L malic = mL NaOH x N NaOH x 0.067 x 200

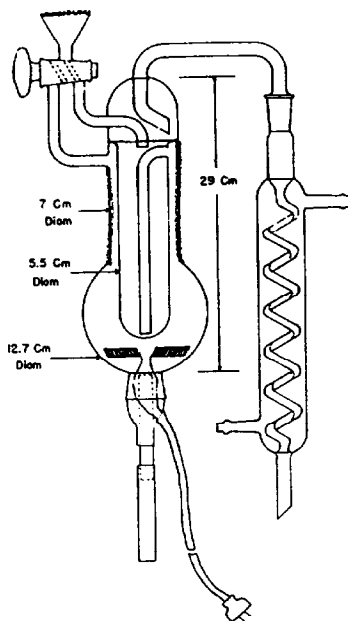
Citrus wines: g/L citric = mL NaOH x N NaOH x 0.064 x 200

**NOTE:** The above calculations are only valid when a 5.0 mL wine sample is used!

## DETERMINATION OF VOLATILE ACIDITY

Distillation of Volatile Acid:

1. Fill the outer chamber of the Cash still about half full of water; make sure that the condenser water supply is on.
2. Turn the stopcock then to the inner chamber filling position. Pipette 10 mL freshly prepared sample into the inner chamber (most wines will require a drop of antifoam) and rinse the sample funnel with about 5 mL distilled water into the inner chamber.
3. Turn the stopcock to the vent position, turn on the heater and allow the outer chamber water to boil.
4. When steam is venting freely, turn the stopcock to the closed position and distill about 100 mL into a beaker.
5. Remove the distillate container; turn the heater off; turn the stopcock to the inner chamber filling position; empty the still by opening the drain tube. While the still is draining, quickly add two 15 mL portions of distilled water through the sample funnel; the draining action will pull these rinses out and clean out the inner chamber for the next sample.



### **Titration of Volatile Acid:**

1. Sample preparation (volatile acid): To the distillate from the volatile acid distillation, add 3 drops phenolphthalein indicator solution.
2. Titrate the sample to a phenolphthalein end point of faint pink with 0.1 N NaOH; record the volume of base used.

### **Calculation:**

*Formula 1:* Volatile acidity (g/100mL) = mL NaOH x N NaOH x 0.6.

**NOTE:** The above calculations are only valid when a 10.0 mL wine sample is used!

Wines with high levels of sulfur dioxide or volatile acidity approaching or exceeding the legal limit should be corrected for sulfur dioxide using one of the following procedures:

a) Add about 0.5 mL of 0.3% H<sub>2</sub>O<sub>2</sub> solution after the sample addition. The hydrogen peroxide solution oxidizes sulfur dioxide and sulfites to nonvolatile sulfate ions, *or*

b) Immediately after titration is complete, cool distillate by holding flask under cold water, add 2-3 mL of starch solution and 1 mL of 1+3 sulfuric acid solution and one crystal of potassium iodide. Rapidly titrate with a 0.002 N iodine solution to a faint blue end point. The quantity of iodine used corresponds to the free sulfurous acid present in the distillate. Record the amount of titrant used to determine free sulfurous acid. For bound sulfurous acid, add 10 mL of 1 N NaOH and boiling beads to the same flask and boil the solution for about 3 minutes. Stopper and cool. Add 2 or 3 mL of starch and acidify with 5 mL of 1+3 sulfuric acid solution and titrate with standardized iodine solution to faint blue end point. The quantity of iodine used corresponds to the amount of bound sulfurous acid. Record the amount of titrant used to determine bound sulfurous acid.

**To calculate the total sulfurous acid as acetic acid, use the following:**

Total the quantities of iodine titrant used in both of the above titrations (free and bound sulfurous acid) in paragraph (b) above and calculate the total sulfurous acid, expressed as g/L of acetic acid, using the following formula:

*Formula 2:*  $\text{SO}_2 \text{ correct} = \text{g}/100\text{mL acetic acid} = \text{mL of I}_2 \times \underline{\text{N}} \text{ of I}_2 \times 6$

**NOTE:** The above calculations are only valid when a 10.0 mL wine sample is used!

The quantity of sulfurous acid, calculated as acetic acid, (*Formula 2*) is then subtracted from the quantity of acetic acid as determined after steam distillation (*Formula 1*, above).

The results of the analysis should be noted as "volatile acid exclusive of sulfur dioxide."

## **FIXED ACIDITY**

Fixed acidity is simply the difference between the total (titratable) acidity and the volatile acidity. As total acidity is expressed in g/L of the principal acid of the fruit from which the wine was made, (e.g., tartaric acid in the case of grape wine) and volatile acid is expressed in g/100mL as acetic acid, the following calculations are necessary to determine the fixed acidity of the wine:

### Grape wines:

Fixed acidity (as tartaric) =

Total acid g/L as tartaric

-(volatile acid g/100mL as acetic acid x 10 x 1.25)

### Apple wines:

Fixed acidity (as malic) =

Total acid g/L as malic

-(volatile acid g/100mL as acetic acid x 10 x 1.12)

### Citrus wines:

Fixed acidity (as citric) =

Total acid g/L as citric

-(volatile acid g/100mL as acetic acid x 10 x 1.17)

Total and fixed acidities are reported in g/L with one decimal place, i.e. X.X g/L, while volatile acid is reported in g/100mL with two decimal places, i.e., 0.XX g/100mL.

### **References:**

1. Ough, C.S. and Amerine, M.A., Methods for Analysis of Musts and Wines, J. Wiley and Son, New York (1988).
2. Official Methods of Analysis of the A.O.A.C. International, Arlington, VA.
3. Zoecklin, B.W., Fugelsang, K.C., Gump, B.H. and Nury, F.S., Production Wine Analysis, Van Nostrand Reinhold, New York (1990).



DEPARTMENT OF THE TREASURY  
BUREAU OF ALCOHOL, TOBACCO AND FIREARMS  
525 Market Street, 34th Floor  
San Francisco, California 94105

REFER TO

April 26, 1983

ATF:RE:A:GN  
5120

Industry Memorandum W-83-09

LABEL STATEMENTS AND ANALYTICAL STATEMENT FOR WINE

To: Proprietors of Bonded Wineries, Bonded Wine Cellars, Taxpaid Wine Bottling Houses and Others Concerned

1. PURPOSE

To clarify the Bureau's position with respect to permissible tolerances for statements on wine labels.

2. BACKGROUND

In recent years it has become increasingly common for wine makers to include analytical information on the label concerning the wine's chemical composition. Statements regarding calories, residual sugar, degree brix of grapes or juice at time of harvest, pH, acid, carbohydrates and fat content are some examples.

3. PROCEDURE

The Bureau has concluded that the following tolerances should be applied when determining the acceptability of statements on wine labels.

A. Caloric Contents

The statement of calories is acceptable provided the actual caloric content of the wine is within the range of +5 and -10 calories of the labeled caloric content.

B. Sugar, pH and Acid Statements

Statements indicating the pH of wine or juice, or the acid contents of juice or wine are acceptable provided the juice or wine contains not more than 10% over or under of the pH or amount of acid stated on the label. Statements indicating residual sugar and degree brix content of the grapes or juice at time of harvest are also acceptable provided the residual sugar does not vary 10% over or under the amount stated on the label, except that for wine with a stated level of 1% residual sugar or less, no tolerance is required.

C. Carbohydrates, Protein and Fat Contents

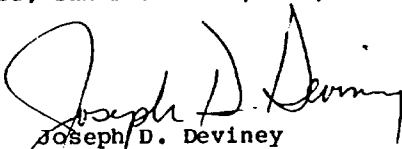
Statements of carbohydrates and fat contents are acceptable provided the actual carbohydrate or fat contents, as determined by ATF analysis, are within a reasonable range below, but in no case more than 20% above, the labeled amount.

Further, statements of protein content are acceptable provided the actual protein content of the wine, as determined by ATF analysis, is within a reasonable range above, but in no case less than 20%, of the labeled amount.

The sole purpose of allowing for these tolerances is to provide for normal production and analytical variables while ensuring that labels are not misleading to the consumer. Proprietors are expected to continue to aim for truthful and accurate statements on labels as specified by 27 CFR 4.38(f).

4. INQUIRIES

Inquiries concerning this memorandum should refer to it by number and be addressed to the Regional Regulatory Administrator, 525 Market Street, 34th Floor, San Francisco, Ca., 94105.

  
Joseph D. Deviney  
Regional Regulatory Administrator

## COMMON CONVERSION FACTORS

### MASS

1,000 kilograms = 1.10 tons

1 kilogram = 1,000 grams = 2.21 pounds

1 pound = 0.454 kilograms = 454 grams

### VOLUME

1 gallon = 3.785 liters = 0.134 cubic feet = 231 cubic inches

1 liter = 0.264 gallons

1 hectoliter = 100 liters = 26.4 gallons

1 cubic foot = 7.48 gallons = 28.3 liters

1 cubic meter = 10 hectoliters = 35.3 cubic feet = 1,000 liters

### CONCENTRATION

1 part per million = 1 milligram per liter  
= 1 gram/1,000 liters  
= 0.00833 pound/1,000 gallons  
= 1 milligram/kilogram

1,000 parts per million = 0.1 percent  
= 1000 milligram/liter  
= 1.0 gram/liter  
= 1.0 part per thousand

1 pound per 1,000 gallons = 120 parts per million  
= 120 grams/1000 liters  
= 0.12 gram/liter  
= 120 mg/liter  
= 0.012 gram/100 mL  
= 0.012 percent



**COMMONLY USED WINE TREATMENT MATERIALS  
and CORRESPONDING THEORETICAL LEVEL INCREASES**

**Potassium Sorbate** (Regulatory limit = 300 ppm; 27 CFR 24.246)

$$\frac{\text{Grams of Potassium Sorbate}}{(.005092 \times \text{gals wine})} = \text{sorbate level (ppm)}$$

Example:

22.43 lbs. of potassium sorbate added to 10,000 gals of wine

22.43 lbs. = 10,183 grams (454 grams per lb.)

$$\frac{10,183}{(.005092 \times 10,000 \text{ gals.})}$$

$$\frac{10,183}{50.92} = 199.7 \text{ ppm (sorbate level)}$$

**Sulfur Dioxide** (Regulatory limit = 350 ppm; 27 CFR 4.22(b)(1))

1. Wine

a. 1 lb. potassium metabisulfite / 1,000 gals. wine = 69 ppm SO<sub>2</sub>

b. 1 lb. liquid sulfur dioxide / 1,000 gals. wine = 120 ppm SO<sub>2</sub>

2. Tons of grapes

a. 1 oz. of potassium metabisulfite / tons grapes = 18 ppm SO<sub>2</sub>

b. 1 oz. of tank (liquid) sulfur dioxide / ton = 30 ppm SO<sub>2</sub>

**Acidulants** (27 CFR 24.182)

1. 1 lb. citric acid / 1,000 gals. wine = TA increase of 0.13 g/L

2. 1 lb. malic acid / 1,000 gals. wine = TA increase of 0.125 g/L

3. 1 lb. tartaric acid / 1,000 gals. wine = TA increase of 0.11 g/L

## Laboratories Certified by ATF as of 1/99 for the Analysis of Wine for the European Union

Beringer Wine Estates  
1000 Pratt Ave. (P.O. Box 111)  
St. Helena, CA 94574

Brown-Forman Beverages Worldwide  
850 Dixie Hwy., P.O. Box 1080  
Louisville, KY 40201

Canandaigua Wine Co., Inc.  
116 Buffalo St.  
Canandaigua, NY 14424-1086

Canandaigua Wine Co., Inc.  
Mission Bell Winery  
12667 Road 24, P.O. Box 99  
Madera, CA 93639

Canandaigua Wine Co., Inc.  
Monterey Cellars  
800 South Alta St., P.O. Box 780  
Gonzales, CA 93926

ETS Laboratories  
899-A Adams St.  
St. Helena, CA 94574

Fetzer Vineyards  
12625 East Side Rd., P.O. Box 611  
Hopland, CA 95449

E. & J. Gallo Winery  
P.O. Box 1130  
Modesto, CA 95353

Gibson Wine Company  
1720 Academy  
Sanger, CA 93657

Glen Ellen Carneros Winery  
21468 Eighth St. East, P.O. Box 1636  
Sonoma, CA 95476

Golden State Vintners  
38558 Road 128, P.O. Box 39  
Sonoma, CA 95476

Heublein, Inc.  
430 New Park Ave.  
West Hartford, CT 06110-1142

Mogen David Wine Corp.  
85 Bourne St., P.O. Box 1  
Westfield, NY 14787

Robert Mondavi Winery  
P.O. Box 106  
Oakville, CA 94562

Mumm Napa Valley  
P.O. Drawer 500  
Rutherford, CA 94573

Northwest Wine Consultants  
509 Merclyn Lane  
Zillah, WA 98953

NYSSA Analytical Laboratories  
141 Suburban Rd., Ste. C-4  
San Luis Obispo, CA 93401

Scott Laboratories, Inc.  
2220 Pine View Way, P.O. Box 4559  
Petaluma, CA 94955-4559

Joseph E. Seagram & Sons, Inc.  
103 Corporate Park Drive  
White Plains, NY 10604-3877

Sebastiani Vineyards  
P.O. Box 1290  
Woodbridge, CA 94258

Silverado Vineyards  
6121 Silverado Trail  
Napa, CA 94558

Stag's Leap Wine Cellars  
5766 Silverado Trail  
Napa, CA 94558

Stimson Lane Vineyards & Estate  
Hwy. 221, P.O. Box 231  
Paterson, WA 99345-0231

Stimson Lane Vineyards & Estate  
14111 Northeast 145<sup>th</sup> st.  
Woodinville, WA 98072

Sutter Home Winery, Inc.  
P.O. Box 248  
St. Helena, CA 94574

United Distillers & Vintners  
430 New Park Ave.  
West Hartford, CT 06110-1142

Vinquiry, Inc.  
7795 Bell Road  
Windsor, CA 95492

The Wine Group  
17000 E. Hwy. 120, P.O. Box 897  
Ripon, CA 95366

**Laboratories Certified by ATF as of 1/99 for the Analysis of Distilled Spirits  
for the European Union**

Barton Brands  
P.O. Box 788, Barton Road  
Bardstown, KY 40004

Jim Beam Brands Company  
Hwy. 245  
Clermont, KY 40110

Brown-Forman Beverages Worldwide  
P.O. Box 1080  
Louisville, KY 40201

Joseph E. Seagram & Sons, Inc.  
103 Corporate Park Drive  
White Plains, NY 10604-3877

E. & J. Gallo Winery  
P.O. Box 1130  
Modesto, CA 95353

Leestown Company, Inc.  
1001 Wilkinson Blvd.  
Frankfort, KY 40601

ETS Laboratories  
899-A Adams St.  
St. Helena, CA 94574

United Distillers & Vintners  
430 New Park Ave.  
West Hartford, CT 06110-1142

Grain Processing Corporation  
1600 Oregon St.  
P.O. Box 359  
Muscatine, IA 52761-0349

United Distillers Manufacturing, Inc.  
3860 Fitzgerald Road  
P.O. Box 740010  
Louisville, KY 40201-7410

Vinquiry, Inc.  
7795 Bell Road  
Windsor, CA 94592

## **Sources of Used Instruments**

**Tom Black Service Center**  
Modesto Office  
(209) 515-3923

**Instrumentation**  
77 – 21<sup>st</sup> Ave., #B  
San Mateo, CA 94403  
(415) 589-3429

**Scientific Equipment Repair Co.**  
667 Rosita Ave.  
Los Altos, CA 94024  
(650) 694-4905

**Transition Equipment Company**  
P.o. Box 108  
Rio Nido, CA 94571  
(707) 869-0396

# SHELF LIFE OF SOME COMMON WINERY LABORATORY REAGENTS

B. A. Bruer, D. R. G. Bruer and C. J. Brien

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## ABSTRACT

Storage times of some common laboratory reagents have been determined. Solutions of 0.057N  $\text{Na}_2\text{S}_2\text{O}_3$  in 0.05N NaOH, 0.1N  $\text{I}_2$ , 0.68N  $\text{K}_2\text{Cr}_2\text{O}_7$  in 12N  $\text{H}_2\text{SO}_4$  and commercial pH 4.01 and 7.00 buffers keep with no apparent deterioration for at least 90 days. The deterioration of the Fehlings A and B solutions stored separately in the dark over a 96 day interval was not chemically significant. Significant deterioration was apparent in

0.1N NaOH, 0.01N NaOH 0.34N  $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4$  in 2N  $\text{H}_2\text{SO}_4$ , Fehlings A and B (stored separately in the light) and the Soxhlet solutions. Deterioration of 0.3%  $\text{H}_2\text{O}_2$  was found after storage for 90 days; however, this decrease would not affect the quantitative recovery of  $\text{SO}_2$  in a wine of 150 mg/L total  $\text{SO}_2$  if the aspiration method of analysis was used.

Standard solutions are required for many analyses routinely performed by quality assurance personnel in wineries. Amerine and Ough (1), Vogel (11), Rankine (8) and A.O.A.C. Methods (2) all comment on the instability or requirement for frequent restandardization of common reagents like  $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4$ ,  $\text{Na}_2\text{S}_2\text{O}_3$ ,  $\text{H}_2\text{O}_2$  and NaOH. During vintage, restandardization may not be practical, and reagents discarded. Fresh solutions are then prepared from standard volumetric concentrates or A.R. reagents. The aim of this study was to characterize changes that occur in concentration of some standard winery laboratory reagents over a period of about three months. Solutions of particular interest were NaOH,  $\text{I}_2$ ,  $\text{H}_2\text{O}_2$ ,  $\text{Na}_2\text{S}_2\text{O}_3$ ,  $\text{K}_2\text{Cr}_2\text{O}_7$ ,  $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4$ , Soxhlet solution, Fehlings solutions and pH buffers 4.01 and 7.00. In addition the effect of some different storage conditions on NaOH,  $\text{H}_2\text{O}_2$ , Soxhlet solution and Fehlings solutions were investigated.

Hydrogen peroxide used in the alkalimetric determination of  $\text{SO}_2$  (9) is present in about 19 times excess for a wine of 150 mg/L total  $\text{SO}_2$ . The excess  $\text{H}_2\text{O}_2$  concentration required for quantitative recovery of  $\text{SO}_2$  was also investigated.

## MATERIALS AND METHODS

Unless specifically stated, all solutions were at  $23^\circ \pm 3^\circ\text{C}$ . The plastic reagent bottles were polyethylene, low density natural colour bottles with wall thickness of 0.7-1.2mm and screw lids. Glass reagent bottles were transparent and sealed with glass stoppers. The dark glass reagent bottles were sealed with screw lids. All solutions except some of the Soxhlet, Fehlings A and Fehlings B solutions were stored in the dark. Analytical grade reagents or commercial standard volumetric concentrates were used for solution preparation, and all solutions were made up in double distilled water. The titrate volumes in the Fehlings/Soxhlet analyses were 20.0mL.

A Radiometer PHM64 meter equipped with a combined electrode was used to measure pH. Further details of storage conditions are shown in Table 1.

To a Roseworthy College dry white wine of about 150 mg/L total  $\text{SO}_2$  was added 100mg/L acetaldehyde and the wine degassed. The  $\text{SO}_2$  was determined using Paul's method (9), except that an aspiration time of 15 minutes was used with a flow rate of 600 to 640 mL per minute. The flow rate was determined using a G.P.E. Meterate gas flow meter. A series of dilutions of the standard  $\text{H}_2\text{O}_2$ , namely 0.31%, 0.16%, 0.078%, 0.042% and 0.021% were made, and the  $\text{SO}_2$  content of the wine was determined at each dilution.

Results were subjected to an analysis of variance, and the sums of squares for differences between the occasions of determination in these analysis further partitioned by fitting either linear or quadratic regressions for concentration on time, similar to Bennett and Franklin (4). For linear and quadratic trends, the time taken for the concentration to decay by 0.5% of the initial concentration was calculated as was the variance of estimated decay time. Further details concerning calculation of the time taken to decay are available from the authors.

## RESULTS

Over the period studied the solutions of sodium thiosulphate (Fig.1), iodine (Fig.2), potassium dichromate (Fig.3) and the commercial pH buffers of pH4 and pH7 showed no deterioration. Fehlings A and B stored in the dark changed at a rate of  $8.6 \times 10^{-3}$  m/L of 0.5% glucose per day (Fig.4). This deterioration was not considered to be chemically significant. All other solutions exhibited significant chemical deteriorations. The estimated decay times and rate of decay of the solutions are shown in Table 2. The decay curves are shown in Figures 5 to 10.

Table 1. Storage conditions of solutions and methods of analysis

Laboratory + Reagent	Volume (L)	Storage Conditions	Period over which analyzed (days)	Number occasions analyzed	Replicates per occasion	Method used to Standardize
0.057N $\text{Na}_2\text{S}_2\text{O}_3$ in 0.05N NaOH	5	dark glass reagent bottle	91	9	10	Vogel (11)
0.1N $\text{I}_2$	1	dark glass reagent bottle	119	9	10	Vogel (11)
0.68N $\text{K}_2\text{Cr}_2\text{O}_7$ in 12N $\text{H}_2\text{SO}_4$	1	dark glass reagent bottle	93	9	10	Vogel (11)
Commercial pH4.01 and pH 7.00 buffers	0.5	Stored in original plastic containers in cupboard	126 (after opening)	8	—	Linnet (7) (against 0.05N $\text{KC}_8\text{H}_5\text{O}_4$ and 0.0825N $\text{Na}_2\text{HPO}_4$ in 0.06N $\text{KH}_2\text{PO}_4$ ) A.O.A.C. (3)
Fehlings A	1.25	(i) transparent glass reagent bottle	96	7	8	
	1.25	(ii) dark glass reagent bottle entirely wrapped in foil	96	7	8	
Fehlings B	1.25	as Fehlings A (i)	96	7	8	A.O.A.C. (3)
	1.25	as Fehlings A (ii)	96	7	8	
Soxhlet	2.5	as Fehlings A (i)	3	7	8	A.O.A.C. (3)
	2.5	as Fehlings A (ii)	3	7	8	
0.1N NaOH	1	glass reagent bottle	93	10	10	Vogel (11)
	1	plastic reagent bottle	93	10	10	
	1	plastic reagent bottle with soda-lime guard tube through lid	93	10	10	
0.01N NaOH	1	plastic reagent bottle	93	10	10	Vogel (11)
0.34N $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4$ in 2N $\text{H}_2\text{SO}_4$	1	glass reagent bottle	93	9	10	Vogel (11)
0.34N $\text{H}_2\text{O}_2$ stabilized with sodium stannate	1	glass reagent bottle 4°	90	9	10	Vogel (11)
	1	glass reagent bottle 23°	90	9	10	
	1	glass reagent bottle 37°	90	9	10	

+ approximate concentrations

The 0.3%  $\text{H}_2\text{O}_2$  may be stored in a glass reagent bottle for 90 days at 4°, 23°, 37°C with a decrease in  $\text{H}_2\text{O}_2$  concentration to 0.29%, 0.26% and 0.23% respectively. Exponential and quadratic curves do not satisfactorily

represent the observed trends (Fig. 11). Results of analysis of a wine of approximately 150 mg/L total  $\text{SO}_2$  a various  $\text{H}_2\text{O}_2$  concentrations showed that  $\text{SO}_2$  recover using 0.042%  $\text{H}_2\text{O}_2$  was significantly different from the

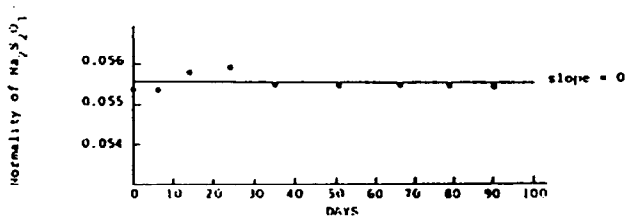


Fig. 1. Change in approximately 0.57N  $\text{Na}_2\text{S}_2\text{O}_3$  with time.

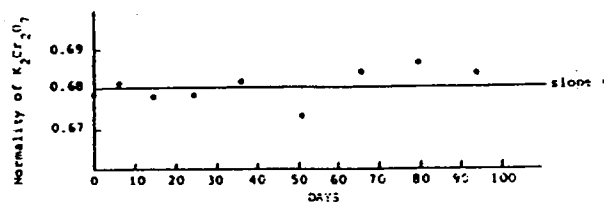


Fig. 3. Change in approximately 0.68N  $\text{K}_2\text{Cr}_2\text{O}_7$  with time.

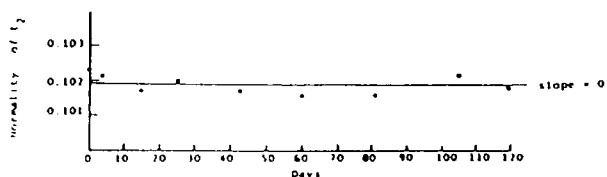


Fig. 2. Change in approximately 0.1N  $\text{I}_2$  with time.

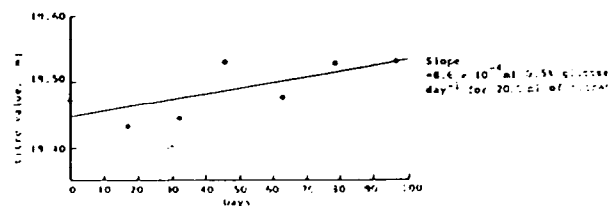


Fig. 4. Change in titre value for separate Fehlings A and B with time. separate Fehlings A and B kept in dark conditions

Laboratory Reagent (Storage condition)	Estimated decay time	Rate of decay
0.1N NaOH (glass bottle)	79 ± 33 days	$6.3 \times 10^{-6}$ N per day
0.1N NaOH (plastic bottle)	44 ± 7 days	$1.1 \times 10^{-5}$ N per day
0.1N NaOH (plastic bottle) and soda-lime guard tube)	31 ± 6 days	$1.6 \times 10^{-5}$ N per day
0.01N NaOH	10 ± 3 days	$4.3 \times 10^{-6}$ N per day
0.34N $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4$ in 2N $\text{H}_2\text{SO}_4$	14 ± 9 days	initial rate $7.9 \times 10^{-5}$ N per day increasing by $5.8 \times 10^{-6}$ N per day
Fehlings A and	75 ± 23 days	$1.3 \times 10^{-3}$ mL of 0.5% glucose per day
Fehlings B (light)	21 ± 1 hours	$4.4 \times 10^{-3}$ mL of 0.5% glucose per hour
Soxhlet (dark)	28 ± 8 hours	$3.4 \times 10^{-3}$ mL of 0.5% glucose per hour
Soxhlet (light)		

at all higher  $\text{H}_2\text{O}_2$  concentrations ( $P < 0.001$ ) (Table 3). The 0.021%  $\text{H}_2\text{O}_2$  can not be compared since the variance for this determination was significantly different ( $P < 0.05$ ) from the variances of the other determinations. As the  $\text{H}_2\text{O}_2$  excess decreased, an increase in standard deviation was observed.

Table 4 lists the levels of significance of regressions

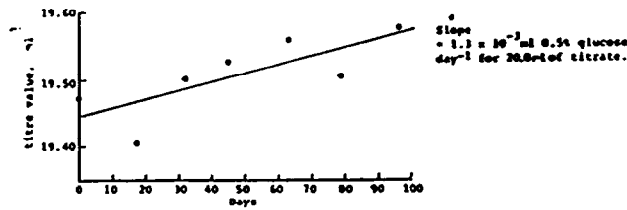


Fig. 5. Change in titre value for separate Fehlings A and B with time, separate Fehlings A and B kept in light conditions.

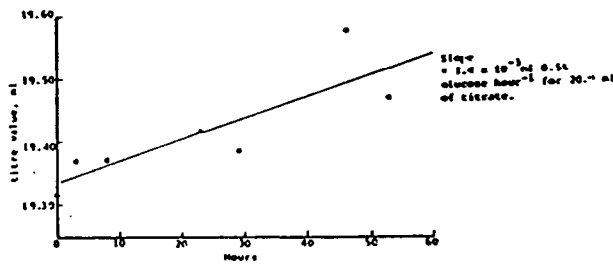


Fig. 6. Change in titre value for Soxhlet solution (mixed Fehlings A and B) with time, Soxhlet solution kept in light conditions.

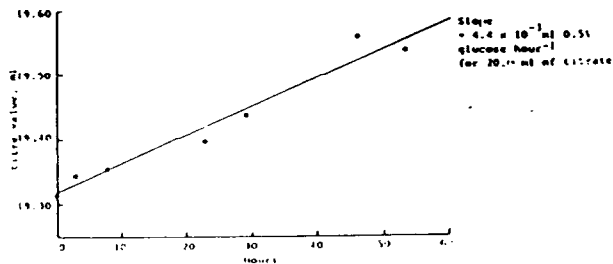


Fig. 7. Change in titre value for Soxhlet solution (mixed Fehlings A and B) with time, Soxhlet solution kept in dark conditions.

and deviations from regression for all analyses.

In most instances, the deviations line was significantly greater than the variability of replicate determinations. Examination of these plots reveals that the points are

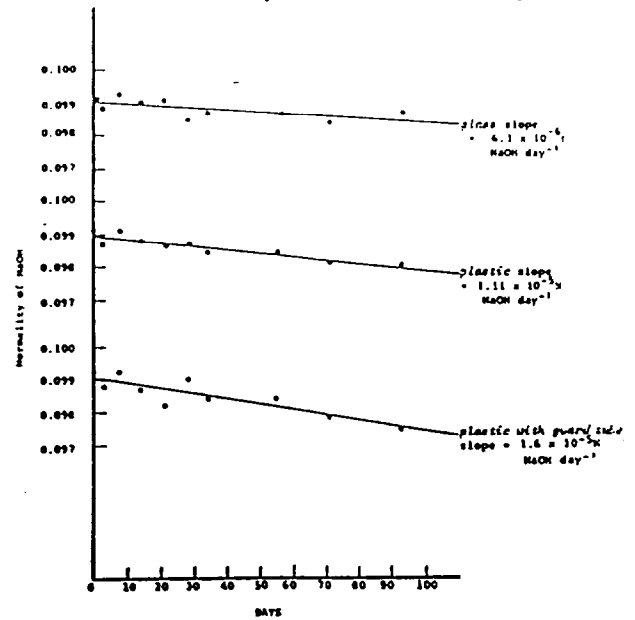


Fig. 8. Change in approximately 0.1N NaOH with time under three different storage conditions.

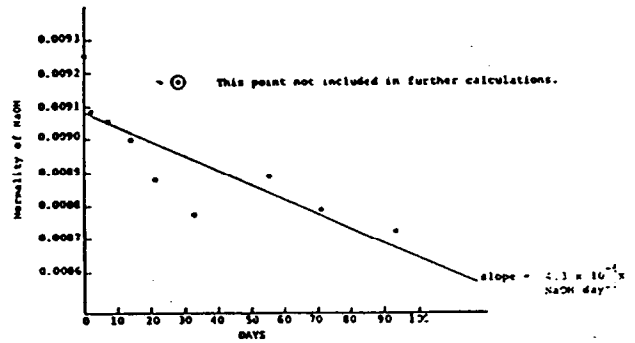


Fig. 9. Change in approximately 0.01N NaOH with time.

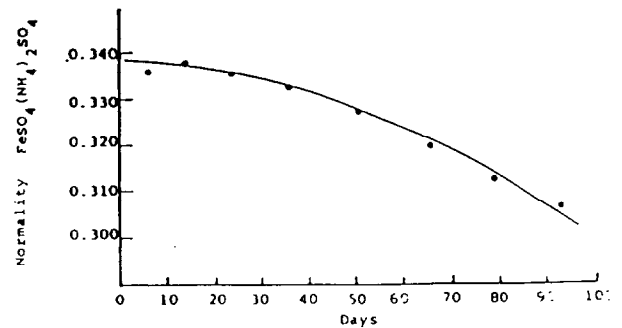


Fig. 10. Change in approximately 0.34N  $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4$  with time.

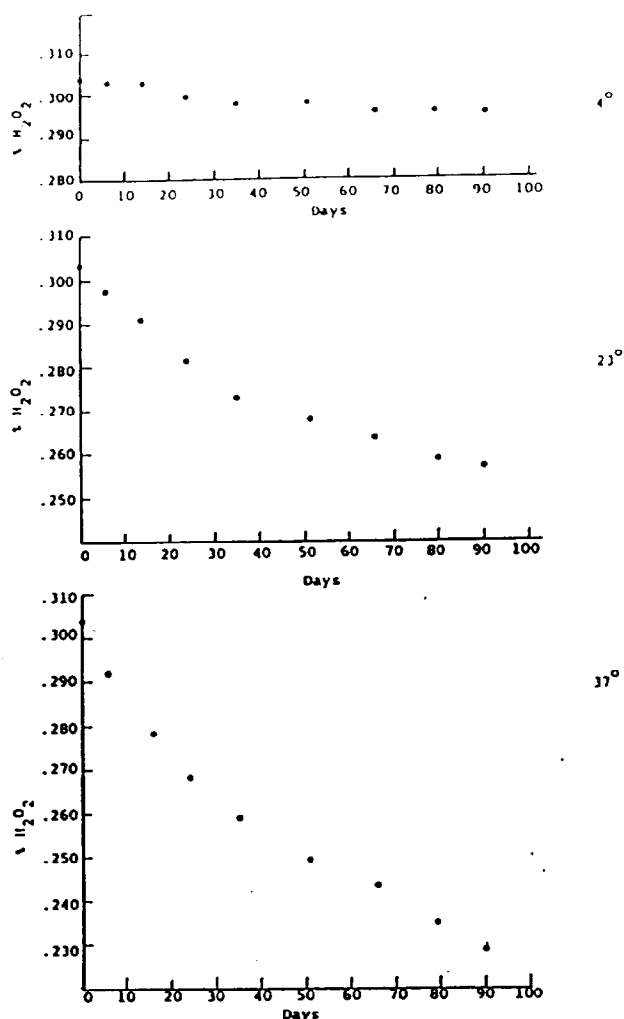


Fig. 11. Change in approximately 0.3%  $H_2O_2$  with time at three temperatures.

distributed haphazardly around the fitted lines. It is concluded that, in cases where the deviations line is significant, this is due to variation in the conditions from time to time which are greater than could be expected from titration variability alone. Comparisons of the means and regressions showed the 0.1N NaOH solution stored in a plastic bottle with a guard tube decayed significantly faster ( $P < 0.001$ ) than 0.1N NaOH stored in glass. Differences between other treatments for 0.1N NaOH were not significant ( $P < 0.05$ ). The results obtained at 90 days for each  $H_2O_2$  solution were analyzed by analysis for variance. The concentration of  $H_2O_2$  was

Table 3. Analysis of total  $SO_2$  at different  $H_2O_2$  concentrations.

$H_2O_2$ concentration (%)	mg/L $SO_2$ recovered	No. of replicates	Std. Dev.
.311	142.5	8	.0238
.156	142.4	8	.0238
.0779	142.0	8	.0311
.0415	140.8	8	.0366
.0208	139.1	8	.0507

Table 4. Coefficient of variation and levels of significance of regressions and deviations for all analyses.

Analyses	Levels of significance		C.V.(%)
	Regression	Deviations	
0.1N NaOH (glass)	c	c	.34
0.1N NaOH (plastic)	c	a	.33
0.1N NaOH (guard tube)	c	c	.36
0.01N NaOH	c	c	.80
$Na_2S_2O_3$	a	c	.13
$I_2$	b	c	.23
$K_2Cr_2O_7$	c	c	.45
$FeSO_4(NH_4)_2SO_4$	c	c	.33
Fehlings A & B (light)	c	c	.30
Fehlings A & B (dark)	b	ns	.34
Soxhlet (light)	c	c	.26
Soxhlet (dark)	c	ns	.25
$H_2O_2$ (at 4°)	—	—	.26
$H_2O_2$ (at 25°)	—	—	.28
$H_2O_2$ (at 35°)	—	—	.27

a/b/c Significant at the 5% level, 1% level and 0.1% level respectively ns not significant, — not applicable.

significantly greater ( $P < 0.001$ ) at 4°C than 23°C, which was in turn significantly greater ( $P < 0.001$ ) than at a 37°C.

## DISCUSSION

Alkaline thiosulfate, iodine and acidified dichromate solutions keep with no apparent decay for at least 90 days. All the other solutions deteriorate with time, some rapidly. If a solution deteriorates, the analyst must ask for how long may the solution be kept. Diamond and Denman (6) state that volumetric analytical procedures are unlikely to be more accurate than  $\pm 0.5\%$ . This is supported by Wildenradt and Caputi (12) who show that it is not unusual for an operator to give results which deviate by more than 0.8% from the mean for the determination of ethanol by dichromate oxidation. An experienced chemist can achieve better results; for the same determination, Caputi (5) found it not unusual for his results to deviate by more than 0.32% to 0.36% of the mean depending on the alcohol concentration. For standardizations carried out in this work, the coefficients of variation ranged from 0.13% to 0.80% depending on the reagent analyzed (Table 4). Youden (13) points out that the accuracy sought and the accuracy attainable vary with the task in hand but "in most analytical work the allowable error lies in the range of  $\pm 0.1\%$  to  $\pm 1.0\%$  for constituents making up more than 1% of the sample". Thus we chose a change in concentration limit of  $\pm 0.5\%$  of the initial value as the useful shelf life of a standard solution, as this will give maximum errors of the same order as those of the operator.

## CONCLUSIONS

This study has shown that alkaline sodium thiosulfate, iodine, and potassium dichromate used respectively in the determination of reducing sugar by the Rebelein method (10), sulfur dioxide by the Ripper method, and ethanol by oxidation, will keep with no apparent deterioration over a three month period. Ferrous ammonium sulphate, used in some methods of ethanol determi-



tion, deteriorates rapidly and should not be stored for more than five days. Sodium hydroxide solutions used for titratable acid determinations keeps for 46 days in glass reagent bottles or 37 days in plastic reagent bottles. Storage with a soda-lime guard tube is not recommended. Dilute sodium hydroxide used in the aspiration sulfur dioxide determination may be stored for seven days in plastic reagent bottles. Separate Fehlings A and B solutions used in the determination of reducing sugar by the Lane-Eynon method, keep with no significant deterioration for three months if stored in the dark. The same solutions will keep for 52 days if stored in the light. Soxhlet solution (mixed equal volumes of Fehlings A and B) will keep 20 hours in light or dark conditions. Commercial pH buffers, of the type normally used to standardize pH meters, keep, on opening, with no apparent deterioration over a four month period. The hydrogen peroxide solution used in the aspiration sulfur dioxide analysis will keep for three months at any temperature likely to be encountered in a winery laboratory during vintage, and still give quantitative recovery of sulfur dioxide at the concentrations normally used in winemaking. Even so, storage of hydrogen peroxide in a refrigerator is recommended.

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